

**AN ANALYSIS OF GILL STRUCTURE OF SELECTED SPECIES IN THE  
GENUS *THRAULODES* (EPHEMEROPTERA: LEPTOPHLEBIIDAE)**

An Undergraduate Research Scholars Thesis

By

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## ABSTRACT

An analysis of gill structure of selected species in the genus *Thraulodes* (Ephemeroptera: Leptophlebiidae) a. (May 2014)

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The genus *Thraulodes* is a common and widely distributed genus of mayflies (Insect: Ephemeroptera) found throughout North and Central America. Larvae are common inhabitants of many different lotic ecosystems throughout the region. Despite their common occurrence, identifying larvae to species can be difficult. One of the primary features in identifying larvae, the length to width ratios of their gills, has been poorly studied and appears to be problematic. The purpose of this research was to take concise and uniform quantitative measures of the gill length to width ratios, and compare these ratios to previously published ratios. No previously published studies have provided data of this detail for any species within this genus. Species included in this study were *Thraulodes brunneus*, *Thraulodes gonzalesi*, *Thraulodes lunatus*, *Thraulodes speciosus*, and *Thraulodes zonalis*. All specimens used in this study are from the Texas A&M University Entomological Collection.

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## NOMENCLATURE

*T*..... *Thraulodes*

EtOH..... Ethanol

# CHAPTER I

## INTRODUCTION

Mayflies are a common and diverse group of aquatic insects found throughout Central America. They are an important component of nutrient cycling and energy flow in these aquatic ecosystems (Reynoldson). Many species of larvae are sensitive to pollution, and changes in the environment, and can often serve as bioindicators of pollution and ecosystem health (Menetrey 2008). Due to their ecological importance, it is imperative that we explore the various species, obtain a large quantity of accurate and relevant data, and translate this data into knowledge. Knowing about these mayfly species in depth can contribute to the success of the species as a whole, as well as how to better preserve their natural habitat. Many species of mayflies have extensive quantities of taxonomic information already gathered and compiled. This compiled data has helped to gain a broad understanding of the order Ephemeroptera as a whole overall. However, there are numerous species that occur in smaller regions and have little data gathered, and are much less known, or that we have vague information on and would contribute greatly from further detailed exploration and study. The species under investigation in this project has extensive morphological data compiled on the adult stages. Though the selected species in this understudy also have extensive information recorded on other life stages, the compilation of this data seems to be much less concise across each species, is vague, and at times apparently inaccurate. The purpose of this research was to clarify taxonomic inaccuracies of selected species of *Thraulodes*. The taxonomy of *Thraulodes* is based predominantly on the adult stage, and the nymph stage has never been formally described (Allen 1978). The first formal descriptions of

*Thraulodes* nymphs were conducted by Needham and Murphy in 1924. It was not until the publications by Traver and Edmunds in 1967 that the nymphal stages of *Thraulodes* were extensively catalogued. Richard K. Allen published generic revisions to *Thraulodes* in 1978, and is the most concise published work on these poorly describes species. Using the Allen paper we will establish a baseline of data, and compare it amongst other publications, as well as in lab gathered data. The majority of *Thraulodes* have a similar flat body, gills, and caudal filaments. The gills of *Thraulodes* are of great taxonomic importance and vary greatly amongst species. Characteristics of gills include round, apex, truncated, broad, narrow, sans lateral trachea, with lateral trachea, and ratios of length to width are of notable importance. In previous attempts to gather taxonomic information on these species either data collected was incomplete or collected in a manner that did not have consistency among specimens. To improve consistency and accuracy for example, a micro ruler was used to ensure that all measurements taken were consistent. We plan on using concise and consistent techniques to take measurements to remedy this issue during this project.

## CHAPTER II

### MATERIALS AND METHODS

#### **Procedures**

Strict consistency when taking measurements was adhered so variations would be minimal. Also, when taking measurements from gills, gill number 4 from the right side was used only in each measurement. This helped increase our consistency and accuracy for every gill ratio taken as compared to taking gill 1 from one specimen then gill 6 from another. Many previous attempts of gathering information on the species has been done in the field, which leads to a larger margin of error, less consistency, and equipment that might not have been as accurate as the equipment found in a lab. To remedy this the same equipment was used each time under the same conditions. For each piece of data collected from each individual specimen, the lab is ensuring that each subsequent specimen as data collected from the same part as the previous specimen. By gathering quantitative data in lab on individual species, we insure that minimal error is incurred. This collected data will then be compared to existing work to ascertain the accuracy and correct errors that might be present in previous works.

Specimens were individually sorted and labeled from existing bulk collections to accurately catalogue each specimen and each measurement taken. Single specimens were then placed in a Petri dish containing 75% EtOH. The individual sample then had the fourth abdominal gill removed from its right side. Once the gill was removed a wet mount slide was created using 75% EtOH as the mounting media. A cover slip was then placed on the slide over the gill to flatten the gill for best measurements. The gill length-width ratio was then recorded using a Nikon SMZ-1 dissecting scope with an attached straight-line reticule with a cross line at

5 micrometers. Each gill was measured at its longest axis and at its widest point using an ocular micrometer. The observed measurements were then recorded. After measurements were collected whole specimens were photographed with a trinocular zoom AmScope using an MU100 AmScope eyepiece camera on ToupView software. The gill that was removed from the specimen was then photographed using AmScope model N-400M on objective 4X using a MU900 AmScope eyepiece camera on ToupView software. Specimens and gill were then returned to individual labeled vials containing 75% EtOH for storage.

### **Equipment**

- 75% EtOH
- Slide Covers
- Wet Mount Slides
- Petri Dish
- Nikon SMZ-1 dissecting Scope
- Ocular micrometer
- Trinocular Zoom AmScope
- MU100 AmScope eyepiece camera
- ToupView software
- AmScope model N-400M
- MU900 AmScope eyepiece camera
- Specimen Vials
- Micro Vials
- Cotton Balls

## CHAPTER III

### RESULTS

#### Species 1: *Thraulodes gonzalesi* TRAVER & EDMUNDS

According to papers published by Traver & Edmunds (1967), Allen (1978) and Baumgardner (2004), this species is generally distributed from Texas, northern regions of Mexico, Arizona, and Guatemala (Baumgardner 2004). The species was described from larvae, male and female adults (Allen, 1978). Mature larvae were reported to have a body length of 9.5-10.5 mm, with narrow gills ranging in a length-to-width ratio from a 7:1- 8:1 (Traver & Edmunds, 1967 and Allen, 1978). Larvae are generally pale with distinctive dark markings (Allen, 1978).

Table 1. Gill Ratios

ID Tag	L	W	Ratio
DB 2010X I I 09 (02)	5.5	1.2	4.58 X1
DB 2011 I to 5 (01)	5.6	1.5	3.73 X1
DB 2010X I I 09(01)	7	1.6	4.3 75X1
DB 96-02	5.1	0.9	5.6 6X1
DB 96-06	4.3	0.8	5.375 X1
DB 98-006 (1999)	3.1	0.7	4.428x1
DB 97-019	4.5	0.7	6.428x1
DB 94-010	3.9	0.6	6.5 X1
DB 01-18	3.5	0.6	5.8 X1
DB 98-006 (2000)	3	0.5	6x1
DB 04-26	3.4	0.7	4.86x1
DB 2010x29(02)	3.4	0.7	4.86x1
DB 2010 X I 19 (01)	4.7	1.2	3.9 X1
DB 04-37	3.8	0.7	5.2 8X1
DB 04-08 25 IV 2004	3.1	0.6	5.1667 X1
DB 99-015 14-v-99	2.6	0.5	5.2 X1
DB 00-03 14-I I I-2000	3.2	0.5	6.2 X1
DB 94-010 -1994	4.2	0.8	5.25 X1
DB 96-02 (01)	2	0.5	4X1

The reported gill ratios for *T. gonzalesi* is 7:1 to 8:1 for middle abdominal gills. However, collected measurements from gill 4 on all specimens indicate a gill length- width ratio much closer to 5:1. Shown in table 1 the extremes in gill length-width ratio were observed at 3.73x1 to 6.5x1. An image of a gill specimen is included for observation in image 1 and 2. This image illustrates the characteristic shape and ratio observed in lab for this species of Thraulodes. Figure 1 shows a graphical representation of the data.

Image 1

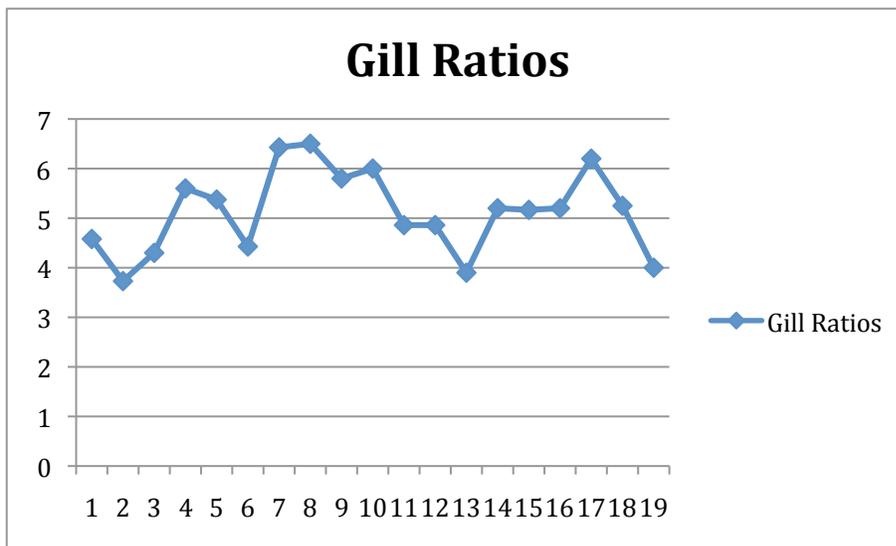


Image 2



DB 2010 X I 19 (01)

Figure 1: Gill Ratios *T. gonzalesi*



**Species 2: *T. speciosus* TRAVER**

According to papers published by Traver & Edmunds (1967), Allen (1978), and Baumgardner (2004), this species is generally distributed in parts of Arizona, New Mexico and parts of Mexico. The species is described from 2 male and 2 female adults (Allen, 1978), and Mayo described the nymphal stage in 1969. Mature larvae were reported to have a body length of 8-9 mm, with wide gills ranging in a length-to-width ratio of 4:1, with nymphs being generally brown to light brown in coloration (Traver and Edmunds, 1967).

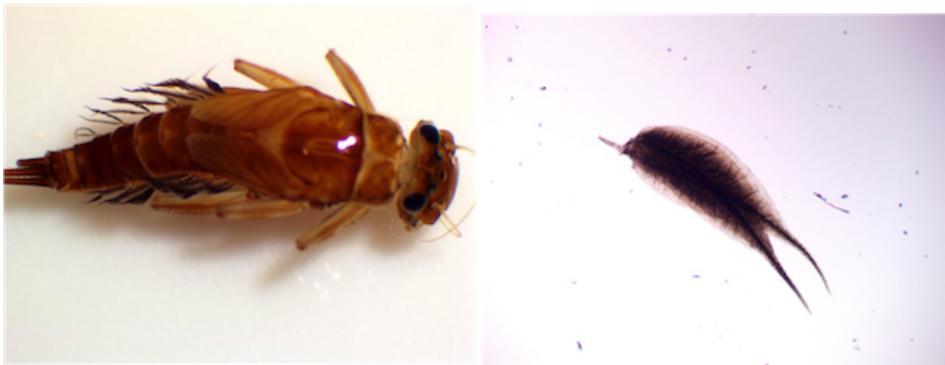
Table 2. Gill ratios

ID Tag	L	W	Ratio
DB 04-22 AZT 27. X. 2012 (01)	6.1	1.6	3.8 X1
DB 04-22 AZT 27. X. 2012 (02)	4.1	1.1	3.7 2X1
DB 04-22 original	2.7	0.6	4.5 X1
DB 04-18 original	3.8	1.1	3.4 5X1
DB 04-30 AZT 27. X. 2012 (01)	4.1	1	4.1 X1
DB 04-30 AZT 27. X. 2012 (02)	3.6	0.8	4.5 X1
DB 04-30 original	4.4	1.1	4X1
DB 04-28 AZT 2.xi.2012 (01)	5.1	1.4	3.6 4X1
DB 04-28AZT 2.X I. 2012 (02)	3.9	1	3.9 X1
DB 04-28AZT 2.X I. 2012 (03)	4.7	1.2	3.9 X1
DB 04-35 DB 2010 .xi.19 (07)	5.9	1.6	3.6 9X1
DB 04-28 AZT 30. X I. 2012 (04)	3	0.7	4.2 8X1
DB 04-35 AZT 30. X I. 2012 (01)	4.6	1.3	3.5 4X1
DB 04-35 AZT 30. X I. 2012 (03)	4.3	1.2	3.58 X1
DB 04-35 AZT 30. X I. 2012 (02)	3.2	0.8	4X1
DB 04-35 AZT 30. X I. 2012 (04)	4.6	1.4	3.29 X1
DB 04-35 AZT 30. X I. 2012 (05)	5.9	1.5	3.9 X1
DB 04-35 AZT 30. X I. 2012 (08)	4.6	1.2	3.8 X1
DB 04-35 AZT 30. X I. 2012 (09)	4.7	1.2	3.92 X1
DB 04-35 AZT 30. X I. 2012 (10)	5.3	1.4	3.79x1

The reported gill ratios for *T. speciosus* is 4:1 for middle abdominal gills in previous works. However, collected measurements in lab from gill 4 on all specimens indicate a gill length- width ratio much closer to 3.86:1 or rounded out to approximately 4:1. Recorded in table 2 for *T. speciosus*, the extremes for gill length-width ratios in lab were observed at 3.29x1 to 4.5x1. All observed in lab measurements for this species fall close to the reported ratio indicating accurate previously documented measurements. Image 3 and 4 are included below for observation of the gill structure and reported ratio. Figure 2 is a graphical representation for this species' data.

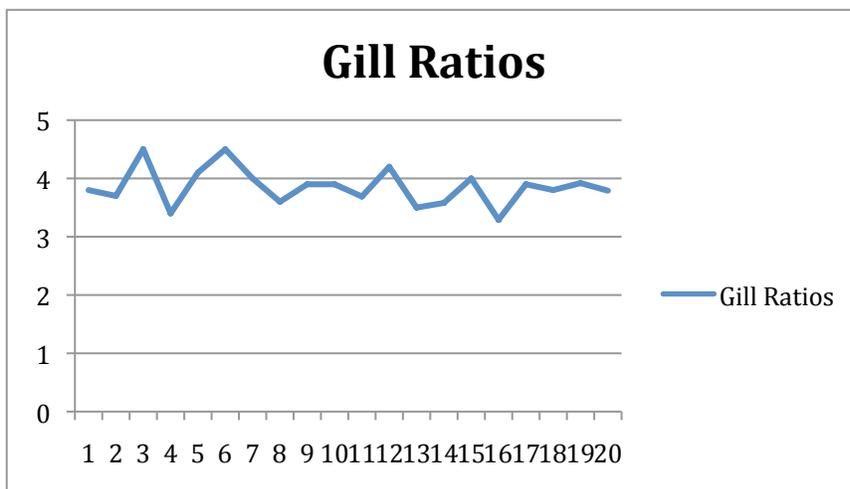
Image 3

Image 4



DB (04–28) AZT 2.xi.2012 (01)

Figure 2: Gill Ratios *T. speciosus*



**Species 3: *T. zonalis* TRAVER & EDMUNDS**

According to papers published by Traver & Edmunds (1967), Allen (1978) and Baumgardner (2004), this species is generally distributed in Central America, from the Canal Zone to Costa Rica. The species was described from a single adult male and a small set of nymphs (Allen, 1978). Mature larvae were reported to have a body length of 8-9 mm, with narrow gills ranging in a length-to-width ratio of 7:1(Traver & Edmunds, 1967). The nymphs are generally brown in coloration with irregular dark markings (Allen, 1978).

Table 3. Gill ratios

ID Tag	L	W	Ratio
08-vi2001 DE Baum. DB 01-23	2.8	0.3	9.33x1
DB 01-23 AZT 4.ii.2013 01	2.7	0.25	10.8x1
DB 2011.ii.15 01	3.2	0.4	8x1
Db 01-32 11.vi.2001 (original)	4	0.6	6.7x1
DB 2011.i.28 (03)	3.5	0.5	7x1
DB 01-32 AZT 4.ii.2013 01	3.4	0.5	6.8x1
DB 01-32 AZT 4.ii.2013 02	3.4	0.4	8.5x1
DB 01-32 AZT 4.ii.2013 03	3.3	0.4	8.25x1
DB 01-26 DB 2011.i.26 (01)	3.7	0.5	7.4x1
DB 01-26 DB 2011.i.28 (01)	3	0.4	7.5x1
DB 01-26 AZT 4.iii.2013 (01)	1.9	0.25	7.6x1
DB 01-26 AZT 4.iii.2013 (02)	2.4	0.3	8x1
DB 01-26 AZT 4.iii.2013 (04)	2.2	0.25	8.8x1
DB 01-26 AZT 4.iii.2013 (03)	2	0.3	6.67x1
DB 01-26 AZT 4.iii.2013 (05)	2.4	0.3	8x1
DB 01-26 AZT 4.iii.2013 (06)	3	0.4	7.5x1
DB 01-53 AZT 4.iii.2013 (01)	2.6	0.4	6.5x1
DB 01-53 AZT 4.iii.2013 (02)	1.8	0.3	6x1
DB 01-53 (original)	2.9	0.4	7.25x1

The reported gill ratios for *T. zonalis* is 7:1 for middle abdominal gills in previous works. Collected measurements in lab from gill 4 on all specimens indicate a gill length- width ratio

close to 7.72:1 or rounded out to approximately 8:1. Recorded in table 3 for *T. zonalis* the extremes for gill length-width ratios in lab were observed at 6x1 to 10.8x1. All observed in lab measurements for this species fall close to the reported ratio indicating accurate previously documented measurements. However, the slight deviation found indicates that further evaluation should be conducted. Image 5 and 6 are included below for observation of the gill structure and reported ratio. Figure 3 is a graphical representation for this species' data.

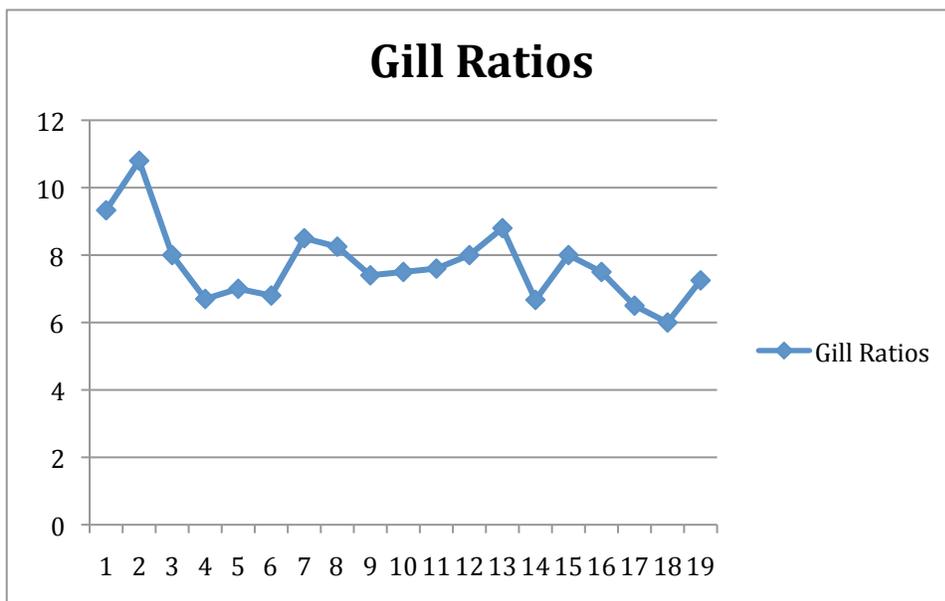
Image 5

Image 6



DB 01-26 DB 2011.i.28 (01)

Figure 3: Gill Ratios *T. zonalis*



**Species 4: *T. brunneus* KOSS**

According to papers published by Traver & Edmunds (1967), Allen (1978) and Baumgardner (2004), this species is generally distributed from central Arizona, SW New Mexico, Honduras, and Mexico. The species was described from a single male (Allen, 1978), and Kilgore and Allen described the nymph in 1973. Mature larvae were reported to have a body length of 7.5-8.5 mm, with narrow gills ranging in a length-to-width ratio from 4:1-5:1 (Traver & Edmunds, 1967). The nymphs are generally brown in coloration with brown and black markings (Allen, 1978).

Table 4. Gill ratios

ID Tag	L	W	Ratio
DB 04-35 AZT 15 iv 2013 (03)	4.3	0.8	5.375x1
DB 04-35 AZT 15 iv 2013 (01)	5.2	1.3	4x1
DB 04-35 AZT 15 iv 2013 (02)	4.5	1.1	4.1x1
DB 04-35 original	3.4	0.7	4.86x1
DB 96-17 original	4	0.9	4.44x1
DB 96-17 AZT 09 x 2013 (01)	3.7	0.8	4.625x1
DB 96-17 AZT 09 x 2013 (02)	4.2	0.9	4.67x1
DB 96-17 AZT 09 x 2013 (03)	4.6	1	4.6x1
DB 96-17 AZT 09 x 2013 (04)	3.7	0.7	5.28x1
DB 01-05 original	5	1.1	4.55x1
DB 01-05 AZT 07 xi 2013 (02)	4.5	0.9	5x1
DB 01-05 AZT 07 xi 2013 (01)	4.1	0.8	5.125x1
DB 97-16 AZT 07 xi 2013 (01)	4.9	1.3	3.77x1
DB 97-16 AZT 07 xi 2013 (02)	3.8	0.8	4.75x1
DB 97-16 AZT 07 xi 2013 (03)	4.6	1	4.6x1
DB 97-16 AZT 07 xi 2013 (04)	4.5	0.9	5x1
DB 97-16 original	3.5	0.8	4.375x1
DB 95-17 AZT 25 ii 2013 (01)	5.5	1.2	4.58x1
DB 95-17 original	4.2	0.9	4.67x1

The reported gill ratios for *T. brunneus* is 4:1-5:1 for middle abdominal gills. Collected measurements in lab from gill 4 on all specimens indicate a gill length- width ratio of 4.65:1 or

rounded out to approximately 5:1 for middle abdominal gills. Recorded in table 4 for *T. brunneus* the extremes for gill length-width ratios in lab were observed at 3.77:1 to 5.375:1. All observed in lab measurements for this species fall close to previously reported data, indicating that measurements in previous works were only slightly misrepresented. Image 7 and 8 are included below for observation of the gill structure and reported ratio. Figure 4 is a graphical representation for this species' data.

Image 7

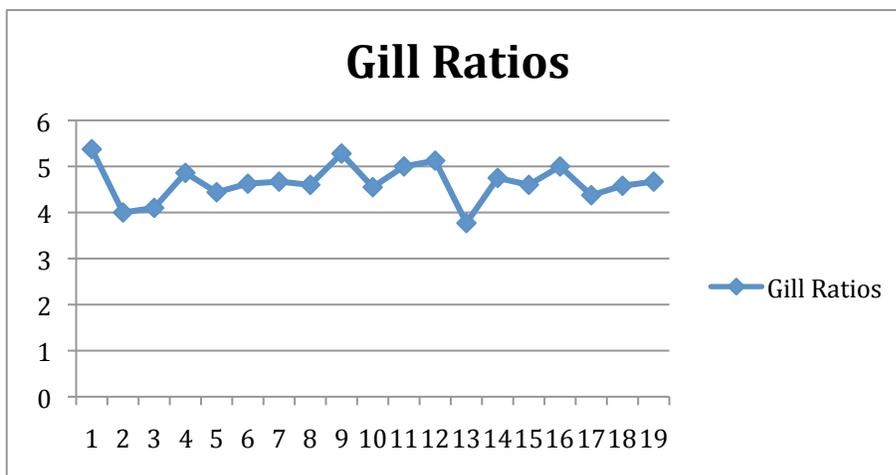


Image 8



17.v.1995 DE Baumgardner (95-17) AZT 18.iii.2013 (01)

Figure 4: Gill Ratios *T. brunneus*



**Species 5: *T. lunates* TRAVER & EDMUNDS**

According to papers published by Traver & Edmunds (1967), Allen (1978) and Baumgardner (2004), this species is generally distributed in northern Mexico. The species is described from a single male and a small set of nymphs from the region (Traver & Edmunds, 1967). Mature larvae were reported to have a body length of 6-7 mm, with narrow gills ranging in a length-to-width ratio from 10:1 (Traver & Edmunds, 1967). The nymphs are generally pale in coloration with brown and black markings (Allen, 1978).

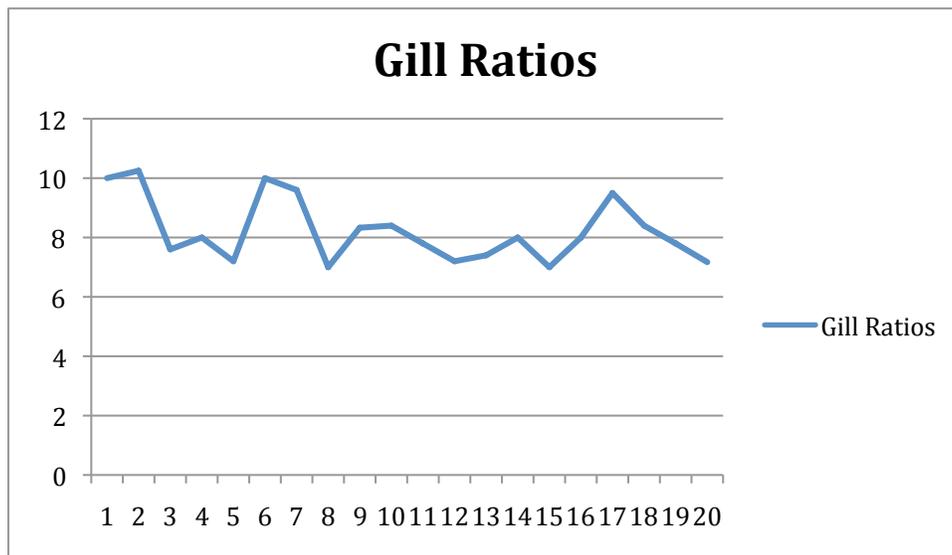
Table 5. Gill ratios

ID Tag	L	W	Ratio
DB (95-11) AZT 18.iii.2013 (01)	4	0.4	10x1
DB (95-11) AZT 18.iii.2013 (02)	4.1	0.4	10.25x1
DB (95-11) AZT 18.iii.2013 (03)	3.8	0.5	7.6x1
DB (95-11) (original)	4	0.5	8x1
DB (95-17) AZT 18.iii.2013 (01)	4.3	0.6	7.2x1
DB (95-17) AZT 18.iii.2013 (02)	5	0.5	10x1
DB (95-17) (original)	4.8	0.6	9.6x1
DB (95-13) DB 2010 xi 19 (03)	3.5	0.5	7x1
DB (95-13) DB 2010 xi 19 (01)	5	0.6	8.33x1
DB (95-13) DB 2010 xi 19 (02)	4.2	0.5	8.4x1
DB (95-13) original	3.9	0.5	7.8x1
DB (95-12) DB 2010 xi 19 (04)	3.6	0.5	7.2x1
DB (95-12) AZT 14 ix 2013 (01)	3.7	0.5	7.4x1
DB (95-12) AZT 14 ix 2013 (02)	3.2	0.4	8x1
DB (95-12) (original)	3.5	0.5	7x1
DB (95-16) AZT 13 ix 2013 (02)	4	0.5	8x1
DB (95-16) AZT 13 ix 2013 (03)	3.8	0.4	9.5x1
DB (95-16) AZT 13 ix 2013 (01)	4.2	0.5	8.4x1
DB (95-16) original	3.9	0.5	7.8x1
DB (95-17) AZT 18.iii.2013 (03)	4.3	0.6	7.17x1

The reported gill ratios for *T. lunatus* is 10:1 for middle abdominal gills. However, collected measurements from gill 4 on all specimens indicate a gill length- width ratio of 8.2325:1 rounded

out to 8:1. Shown in table 1 the extremes in gill length-width ratio were observed at 7x1 to 10.25x1. This deviation indicates inaccuracy in previous measurements and should require further study. An image of a gill specimen is included for observation in image 5. This image illustrates the characteristic shape and ratio observed in lab for this species of *Thraulodes*. Image 9 and 10 will be included below for observation of the gill structure and reported ratio. Figure 5 is a graphical representation for this species' data.

Figure 5: Gill Ratios *T. lunates*



## CHAPTER IV

### CONCLUSION

Nymphs having a similar distinctive abdominal pattern are associated with this species, even though this relationship has not been confirmed by rearing for all species present (Traver & Edmunds 1967). Each nymph observed in lab was compared to existing field and lab data. To test the accuracy of previous measurements, as it was believed that not all data previously collected was accurate. Much of the work previously done was in the field and could not obtain optimum accuracy. By using consistent gills from each specimen as well as a consistent measuring method, collected data in lab is considered accurate and consistent.

When compared to the existing data we were able to confirm both the gill length-width ratios of *T. speciosus* and *T. zonalis* at 4x1 and 7x1 respectively. We also showed that the preexisting reported ratio of 7x1-8x1 for *T. gonzalesi* was inaccurate, and is actually much closer to a length-width ratio of 5x1. The preliminary findings for *T. brunneus* indicate a gill length-width ratio of approximately 9x1. This only differs slightly from previously recorded individuals of this species, which were reported a middle abdominal gill ratio of 10x1. It is suggested to rear specimens in lab to have even more accurate results as well as specimens all of the same age so as to ensure gill ratios do not have such extremes. These extremes, and slight variations in some species, are believed to have been caused by measurements gathered from immature nymphs and being compared it to mature nymphs, which might have slightly differing gill ratios through development. Another hypothesized contributing factor for reported and collected ratios differing

is the possibility that previously reported ratios did not use a consistent gill, as well as sampling error due to in field observations.

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